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Chromate Inhibition of Metabolism by Rat Tracheal Explants. II. In vivo Exposures

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16. ABSTRACT

We have previously shown that incubation of rat tracheal slices in tissue culture medium containing 0.4-2.6mm (mmol/liter) Na_2CrO_4 causes inhibition of secretion of mucus glycoproteins by such explants. At concentrations of Na_2CrO_4 of 0.27mm and above, the inhibition of secretion rate could be correlated with damage to the tracheal epithelium evaluated by histopathologic observations. In the present study we extend these findings by determination of the effects of Na_2CrO_4, administered to rats in vivo (for 24 hr) as an aerosol with a mass median aerodynamic diameter of 1-2 μm at concentrations of 0.051 and 0.173 mmol/m^3, upon the previously evaluated parameters. With the aid of several simplifying assumptions, the in vivo dosages of Na_2CrO_4 can be compared with the equivalent concentrations to which trachaea were exposed in vitro in the earlier study. When such calculations are made, there is an excellent correlation between the toxicity observed at equivalent dosages of Na_2CrO_4 in vivo and in vitro. Such data may be used to calculate the tracheal deposition factor, F, the percentage of the total Na_2CrO_4 mass from the inhaled aerosol deposited upon the trachea. The value of F so obtained, 0.4-0.7%, compares favorably with results calculated previously on the basis of deposition of radiolabeled fused aluminosilicate particles. Thus, the tracheal explant technique may prove useful as a dosimeter for studying the quantitative in vivo deposition of freely diffusable, water-soluble agents on various airway surfaces of experimental animals.

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Chromate Inhibition of Metabolism by Rat Tracheal Explants II. *In Vivo* Exposures

JEROLD A. LAST, OTTO G. RAABE, PETER F. MOORE, AND BRIAN K. TARKINGTON

California Primate Research Center, University of California, Davis, California 95616

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Chromate Inhibition of Metabolism by Rat Tracheal Explants. II. *In Vivo* Exposures. LAST, J. A., RAABE, O. G. MOORE, P. F., AND TARKINGTON, B. K. (1979). *Toxicol. Appl. Pharmacol.* 47, 313–322. We have previously shown that incubation of rat tracheal slices in tissue culture medium containing 0.4–2.6 mM (mmol/liter) Na_2CrO_4 causes inhibition of secretion of mucus glycoproteins by such explants. At concentrations of Na_2CrO_4 of 0.27 mM and above, the inhibition of secretion rate could be correlated with damage to the tracheal epithelium evaluated by histopathologic observations. In the present study we extend these findings by determination of the effects of Na_2CrO_4, administered to rats *in vivo* (for 24 hr) as an aerosol with a mass median aerodynamic diameter of 1–2 μm at concentrations of 0.051 and 0.173 mmol/m^3, upon the previously evaluated parameters. With the aid of several simplifying assumptions, the *in vivo* dosages of Na_2CrO_4 can be compared with the equivalent concentrations to which trachaea were exposed *in vitro* in the earlier study. When such calculations are made, there is an excellent correlation between the toxicity observed at equivalent dosages of Na_2CrO_4 *in vivo* and *in vitro*. Such data may be used to calculate the tracheal deposition factor, *F*, the percentage of the total Na_2CrO_4 mass from the inhaled aerosol deposited upon the trachea. The value of *F* so obtained, 0.4–0.7%, compares favorably with results calculated previously on the basis of deposition of radiolabeled fused aluminosilicate particles. Thus, the tracheal explant technique may prove useful as a dosimeter for studying the quantitative *in vivo* deposition of freely diffusable, water-soluble agents on various airway surfaces of experimental animals.

In an earlier study (Last *et al.*, 1977), we demonstrated that the secretion of mucus glycoproteins by tracheal slices incubated for 24 hr in tissue culture is inhibited by inclusion of Na_2CrO_4 in the culture medium. The extent of such inhibition was dependent upon the concentration of chromate in the medium; a biphasic dose–response curve was observed. In the second phase, at higher concentrations of chromate (above 0.27 mM), we also observed inhibition of precursor [^{3}H]glucosamine uptake and gross cytotoxicity. In the present study, analogous studies have been performed with tracheal explants from rats exposed to Na_2CrO_4 *in vivo*, administered as an aerosol. We find that for equivalent concentrations of Na_2CrO_4 administered *in vivo* and *in vitro* (calculated as described below), there is equivalent inhibition of glycoprotein secretion rate in the tracheal explant assay (Last *et al.*, 1977). In addition, we have extended the previous findings of chromate-induced cytotoxicity by histochemical staining techniques.

Such a quantitative comparison of pollutant effects *in vivo* and *in vitro* is, to the best of our knowledge, a completely novel approach with no published precedents.

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$CaCrO_4$ has been administered to mice *in vivo* as a dust (Nettesheim and Szakal, 1972). This experiment was a chronic exposure (18 months), and the focus of study was carcinogenesis and changes in the alveoli. An incidental observation was epithelial cell necrosis in large airways (Nettesheim and Szakal, 1972).

METHODS

Rats were chronic respiratory disease-free males, derived from the Sprague–Dawley strain, purchased from Hilltop (HLA-SD). All rats within a given experiment were matched as to age (70–80 days) and handling procedures. After 24 hr of exposure to the chromate aerosol, generated as described below, rats were removed from the chambers and killed by pentobarbital overdose and exsanguination. Tracheae were removed and processed exactly as described previously (Last *et al.*, 1977) for quantitation of secretion rate.

Slices from the laryngeal end of the tracheae were fixed in modified Karnovsky's fixative (Mellick *et al.*, 1977) for 24 hr, dehydrated in ethanol, and embedded in glycol methacrylate. Subsequently, 1- to 2-μm sections were cut with glass knives on a JB-4 (Sorvall) microtome. Sections for routine morphological evaluation were stained with toluidine blue. Specific stains used to evaluate mucosubstances were alcian blue–periodic acid–Schiff at pH 1.0 and 2.5 (Jones and Reid, 1973).

Na_2CrO_4 aerosols were generated as shown schematically in Fig. 1. Solutions of sodium chromate in distilled water were nebulized with a Babington type nebulizer ("Solo-Sphere," McGaw Respiratory Therapy). The nebulizer was immersed in an ice-water bath during operation to reduce water evaporation from the solution. The aerosol was diluted with clean, dry air immediately downstream from the nebulizer to reduce the relative humidity. A ^{85}Kr discharger was used to reduce the aerosol electrostatic charge to Boltzmann equilibrium. The discharger was heated slightly with a heating tape to enhance droplet drying. The conditioned aerosol was then introduced into the inlet air stream of a 450-liter exposure chamber (Hinners *et al.*, 1968). The chamber volumetric flow rate was 225 liters/min (30 air changes per hr). Chamber relative humidity did not exceed 60% at 22°C.

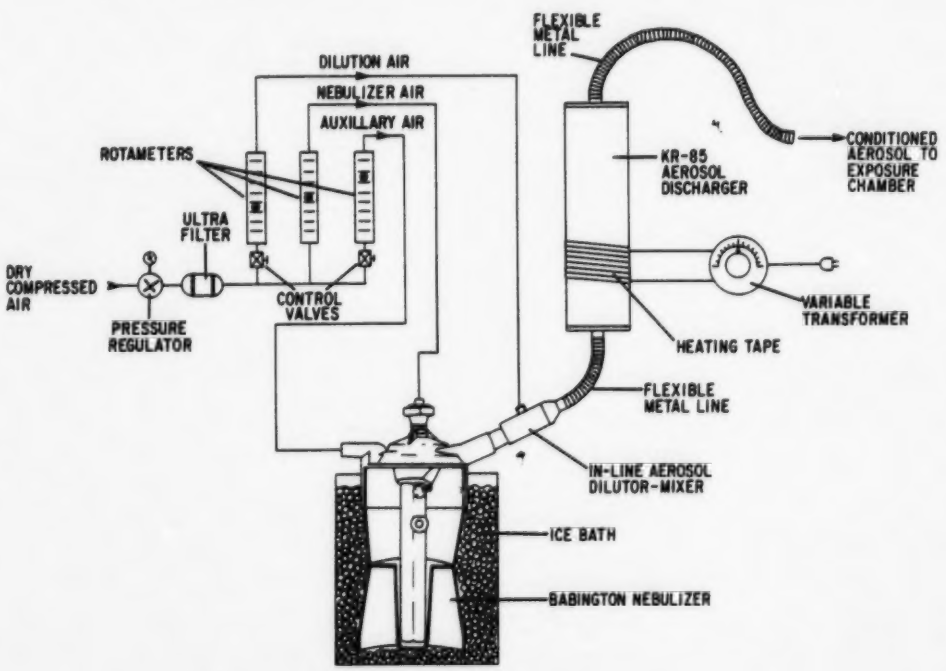

FIG. 1. Schematic diagram of sodium chromate aerosol generation system.

RESULTS

Aerosol Generation

Before we performed the exposures we made the following assumptions for planning purposes. The concentration of a sodium chromate solution required to produce an aerosol in the chamber with a mass median aerodynamic diameter (MMAD) of 1.75 μm (small enough to penetrate the nasal defenses, large enough to ensure significant tracheal deposition) was calculated using the aerodynamic resistance diameter defined by Raabe (1976). Assuming a density of 1.483 for sodium chromate (decahydrate) particles, an aerosol with a mass median geometric diameter (MMD) of 1.36 μm would have an MMAD of 1.75 μm. A nebulizer concentration of 0.17 M required to yield this size distribution was calculated as given by Raabe (1975) by use of the equation:

$$C_N = \frac{\rho(\mathrm{MMD})^3}{(\mathrm{VMD})^3} \qquad (1)$$

with ρ the particle density, C_N the solution concentration in g/cm^3, MMD the particle mass median diameter, and VMD the volume median diameter of the droplets produced by the nebulizer (in micrometers). The VMD of the droplets produced by the Babington nebulizer was assumed to be 4 μm (Raabe, 1975). Thus Eq. (1) gives:

$$C_N\,(\mathrm{g/cm^3}) = \frac{\rho(\mathrm{MMD})^3}{(\mathrm{VMD})^3} = \frac{(1.483\ \mathrm{g})(1.36\ \mu\mathrm{m})^3}{(\mathrm{cm^3})\quad(4\ \mu\mathrm{m})^3}$$

$$= \frac{0.0582\ \mathrm{g}}{\mathrm{cm^3}} = \frac{58\ \mathrm{g/liter}}{342\ \mathrm{g/mol}}$$

$$= 0.17\ \mathrm{mol/liter}.$$

Air at 1.41 kg/cm^2 supplied to the nebulizer yielded an aerosol output of 26 μl/liter in 7.7 liters/min of air flow, or 200 μl/min. This multiplied by the molar concentration, divided by the total air flow through the chamber, yields 0.151 mmol/m^3 as the theoretical concentration. The measured aerosol mass concentration of 0.051 mmol/m^3 (17 mg/m^3 for the sodium chromate decahydrate particles) in the exposure chamber indicated that the system losses were about 66% of the nebulized sodium chromate. A higher mass concentration of 0.173 mmol/m^3 (59 mg/m^3) in the chamber was achieved by the addition of 24 liters/min of auxiliary air to the 7.7 liters/min of air flow through the nebulizer. The aerosol output with auxiliary air was then 17 μl/liter in 32 liters/min of air flow; about 50% of the mass of sodium chromate nebulized was lost in the system with auxiliary air flow.

The actual exposure data for the two exposure levels are summarized in Table 1. Chamber aerosol mass concentrations were measured by collection of aerosol samples from known volumes of air on glass-fiber filters (Type AE, Gelman Instrument Company) and by weighing the filters before and after collection to determine their increase in mass. The aerodynamic size distributions were determined with a seven-stage cascade impactor (ARIES Model 04-002) and subsequent atomic absorption analysis of the chromium on each stage and on the back-up filter. A log-normal distribution was fitted to each sample set of data and the values reported are the MMAD and geometric standard deviation (σ_g) for each fitted distribution.

Comparison of the chromium concentration obtained by atomic absorption analysis of the impactor samples and the aerosol mass determined by filter sample weights showed the sodium chromate aerosol to be in the form of the decahydrate. The somewhat smaller median sizes observed during the exposures than predicted from Eq. (1) may be related to losses of large particles in the aerosol generation and delivery system and/or to smaller droplets than assumed being produced from the nebulizer.

Calculation of the relationship between aerosol concentrations for *in vivo* exposures and chromate concentrations in tissue culture medium *in vitro* was performed as follows. An aerosol depositing on the trachea of a rat is

TABLE 1

EXPOSURE OF RATS TO CHROMATE AEROSOL: EXPOSURE DATA

Exposure number	Aerosol concentration (mean ± SE)			Aerodynamic size[a]	
	From filter sample		From cascade impactor	Mass median aerodynamic	
	mg/m³	mmol/m³	(mmol/m³)	diameter (μm ± SE)	$\sigma_g \pm$ SE
1	17.3 ± 0.3[b]	0.051	0.058	1.73 ± 0.16	1.47 ± 0.08
2	59.0 ± 3.2[c]	0.173	0.159 ± 0.010	1.29 ± 0.21	1.86 ± 0.14

[a] Data from a fitted log-normal distribution using a weighted least-squares program.
[b] $n = 2$.
[c] $n = 6$.

cleared with a half-time, T, and a rate constant, L. If A is the amount of aerosol (Na_2CrO_4) in the trachea at a given time, then the change in this amount is equal to the amount depositing in the trachea per unit time, D, minus that cleared:

$$dA/dt = D - LA \tag{2}$$

which may be solved as:

$$A = D/L(1 - e^{-Lt}) \tag{3}$$

where t is the time after beginning the exposures. If $Lt \gg 3$, the amount of Na_2CrO_4 in the trachea is at an equilibrium value:

$$A = D/L. \tag{4}$$

A reasonable tracheal clearance rate for rats is a half-time of about 4 min (Bang and Bang, 1977), thus L is about 0.693/4 or 0.17 min^{-1} and $Lt \gg 3$ for exposures longer than 17 min.

An additional potential complication is clearance via the trachea of N_2CrO_4 deposited in the bronchial tree, which will increase the level and duration of residence of Na_2CrO_4 in the trachea. The solubility of Na_2CrO_4 in body fluids will have an unknown (but presumably constant) effect on reducing the contribution to the amount of Na_2CrO_4 in the trachea from bronchial clearance. If the concentration of Na_2CrO_4 in the trachea is C, and the total volume of fluid and tissue containing Na_2CrO_4 is V, then:

$$C = A/V \tag{5}$$

and the deposition rate is:

$$D = LA = LCV. \tag{6}$$

The deposition rate is also given by:

$$D = C_A V_m F = LCV \tag{7}$$

where C_A is the mass of Na_2CrO_4 per unit volume of air, V_m is the minute volume for a rat (about 150 ml for a 250-g rat), and F is the deposition fraction. We assumed for planning purposes (Raabe *et al.*, 1977) a value for F of 0.4% (based on the aerodynamic diameter of the particles) and a tracheal fluid volume, V, of 4×10^{-3} ml (Yoneda, 1976):

$$C_A = LCV/V_m F = \frac{(0.17)(4 \times 10^{-3})C}{(150)(4 \times 10^{-3})}$$

$$= 1.13C \text{ (mmol/m}^3\text{)} \tag{8}$$

where C is the desired concentration in the fluid bathing the trachea, in (mmol/liter). Hence, C could be calculated by:

$$C = 0.882C_A \tag{9}$$

For example, at $C_A = 0.058$ mmol/m³, the predicted concentration in the tracheal fluid would be 0.051 mmol/liter at equilibrium.

On the other hand, we can omit assumptions about the value of F in Eq. (8), and express the relationship between C_A and C as follows:

$$C_A = LCV/V_m F = \frac{(0.17)(4 \times 10^{-3})(C)}{(150)(F)}$$

$$= 0.452\, C/F \text{ (mmol/m}^3\text{)} \tag{10}$$

where C_A is the aerosol concentration in mmol/m³, C is the desired tracheal fluid concentration in mmol/liter, and F is the deposition fraction as the percentage of inhaled aerosol. Hence, if C and C_A are known, the effective deposition fraction can be calculated by:

$$F = 0.452\, C/C_A(\%). \qquad (11)$$

For example, if $C_A = 0.06$ mmol/m³ and the tracheal concentration C is 0.07 mmol/liter, the deposition fraction would be 0.53%.

[³H]Glucosamine Incorporation

Tracheal slices from rats exposed to Na_2CrO_4 aerosol *in vivo* were incubated with [³H]glucosamine for 24 hr in tissue culture medium. The rate of mucus secretion was then evaluated as the trichloroacetic acid-precipitable radioactivity in the culture medium. As shown in Table 2, exposure to the Na_2CrO_4 aerosol reduced the subsequent capacity of tracheal slices to secrete mucus glycoproteins in tissue culture. At 0.173 mmol/m³ the apparent inhibition of secretion was about 57%, a significant decrease. The reduction of about 29% at 0.015 mmol/m³ was also statistically significant. These results can be compared with our previous data, obtained by incubation of tracheal slices *in vitro* with Na_2CrO_4, by use of Eq. (8). If we assume a tracheal deposition fraction, F, of 0.4% based on previous work with insoluble particles of the same approximate size as the aerosols used in the present study (Raabe *et al.*, 1977), then we can calculate what the apparent concentration of Na_2CrO_4 dissolved in the fluid bathing the tracheal surface [C, Eq. (8)] must have been during the exposure. The values thus obtained can be compared with our earlier *in vitro* data and the percentage inhibitions of glycoprotein rate thus obtained may also be compared. When such calculations were actually performed, we found values of 79 and 44% of the control secretion rate at *in vitro* concentrations analogous to 0.051 and 0.173 mmol/m³ aerosol concentrations *in vivo*, respectively. These values may be compared with those actually observed, as given in Table 2, and may be seen to agree very satisfactorily with the observed values. Considering the nature of the assumptions involved in calculating *in vivo* dosages from *in vitro* data, the agreement between the two sets of data is striking. It is appropriate to point out that two modes of action of Na_2CrO_4 were described in the earlier paper (Last *et al.*, 1977), and that 0.051 mmol/m³ of Na_2CrO_4 is below the threshold level for observation of gross morphological damage, while 0.173 mmol/m³ of Na_2CrO_4 was as close as our apparatus could come to the minimal level at which frank damage was observed (which is why

TABLE 2

EFFECT OF CHROMATE EXPOSURE *in vitro* AND OF CHROMATE AEROSOL EXPOSURE *in vivo* ON GLYCOPROTEIN SECRETION BY RAT TRACHEAL EXPLANTS

Exposure number	Chromate aerosol concentration (mmol/m³)	Glycoprotein secretion rate (% of control value)	*In vitro* concentration (mM) of chromate giving equivalent inhibition[a]	Apparent tracheal deposition fraction, F (%)
1	0	100	—	
	0.051	71[b]	0.045	0.40
2	0	100	—	
	0.173	43[c]	0.245	0.64

[a] Data from Last *et al.*, 1977.
[b] $p < 0.0125$ by Student's t test ($n = 12$).
[c] $p < 0.0005$ by Student's t test ($n = 16$).

these two concentrations were chosen for this study).

On the other hand, we can avoid making any assumptions about the value of F and use Eq. (11), as we have done in Table 2. In this case, we assumed that the concentration of Na_2CrO_4 in the fluid bathing the tracheal surface *in vivo* could be calculated as being the same as the concentration required to elicit the equivalent percentage inhibition of glycoprotein secretion rate *in vitro*. With C and C_A [Eq. (11)] both known, the value of F could be calculated, as shown in Table 2. In this case the agreement between intra-experimental values and between interexperimental values (Raabe *et al.*, 1977) is very satisfactory.

Other Biochemical Assays

In addition to the experimental data of Table 2, we have utilized various assays of lung homogenates prepared from rats exposed to Na_2CrO_4 aerosols to evaluate the generality of the responses observed. In particular, we evaluated pulmonary edema by measurement of lung wet-to-dry-weight ratios. Concurrently, we estimated inflammatory and reparative responses in the distal lung by measurements of RNA, DNA, and protein levels of such lung homogenates. In brief, we observed statistically significant ($p < 0.05$, Student's one-tailed t test) elevations in all of these parameters evaluated at the 0.173 mmol/m^3 level of chromate aerosol. We observed similar trends (not significant) in these determinations at the lower level of chromate aerosol (0.051 mmol/m^3) evaluated. Thus, we can conclude that enough of the chromate aerosol was present as smaller sized particles to lead to significant deposition in the distal airways, even though clearance from these distal airways to the trachea was apparently not a major component contributing to Na_2CrO_4 deposition in the trachea.

Histology

Routine morphological evaluations of sections from tracheae of the same rats from which the data of Table 2 were acquired showed no apparent differences between control animals and those exposed to 0.051 mmol/m^3 of Na_2CrO_4 aerosol. Limited histochemical staining evaluations also gave an impression of there being no difference between control and exposed rat trachea. At 0.173 mmol/m^3 of Na_2CrO_4 aerosol there was marked disorganization of the tracheal epithelium, as shown in Figs. 2 and 3.

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FIG. 2. Surface epithelium of a normal rat trachea, stained with toluidene blue, × 384.

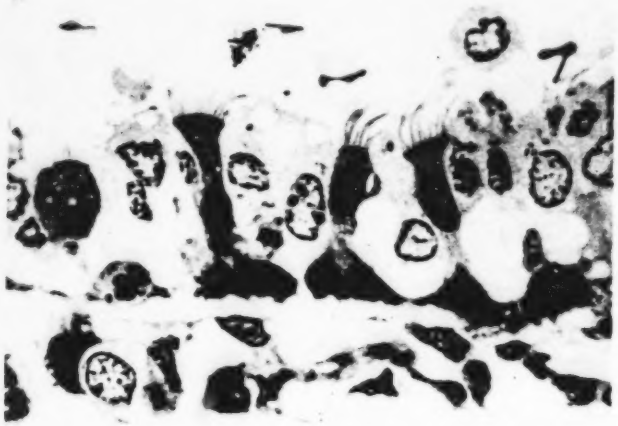

FIG. 3. Surface epithelium of a trachea from a rat exposed for 24 hr to 0.173 mmol/m³ of Na_2CrO_4 aerosol, stained with toluidene blue, × 384.

Numerous foci of epithelial necrosis and ulceration were present. In places where the epithelium was still intact, there was prominent intercellular edema. Both the nonciliated secretory cells and the ciliated cells had condensed and marginated chromatin. The cytoplasm of ciliated cells often had an increased affinity for the stain. The apical granules of the nonciliated secretory cells were less evident and the cytoplasm of these cells was swollen and stained less densely than similar cells from control tracheae. Neutrophils and mononuclear inflammatory cells had infiltrated the submucosa and were also abundant in the foci of epithelial ulceration. Degenerative changes were also present in the secretory cells of the submucosal glands. The changes were comparable qualitatively to, but were less extensive than, the changes in the surface epithelial cells. Such changes are consistent with those previously observed after exposure to Na_2CrO_4 *in vitro* (Last *et al.*, 1977).

Histochemical evaluation of control rat tracheae and tracheae from rats exposed to 0.173 mmol/m³ of Na_2CrO_4 aerosol showed some additional striking differences. In Fig. 4 is shown a control rat trachea stained with alcian blue–PAS at pH 2.5. The most abundant secretory cell type in the surface epithelium is the small nonciliated secretory cell (also known as a serous cell [Jeffery and Reid, 1977]); this cell produces mainly neutral mucosubstances. The large nonciliated secretory cell (or true goblet cell) is infrequently encountered in the airway epithelium of chronic respiratory disease-free rats. The secretory cells of the submucosal glands produce neutral and acidic mucosubstances, either singly or in combination in any particular cell. The acidic mucosubstances are probably predominantly sialomucins, based on the observation that sialomucins are not alcianophilic if stained with alcian blue at pH 1.0 (Jones and Reid, 1973).

When similar histochemical evaluations are performed on tracheal sections from rats exposed to 0.173 mmol/m³ of chromate aerosol, the results shown in Fig. 5 are obtained. Chromate exposure resulted in focal necrosis and ulceration of the tracheal epithelium. In places where the epithelium was still intact, there was a marked decrease in stainable mucosubstance in the small nonciliated secretory cells. The large nonciliated secretory cells (true goblet cells) did not

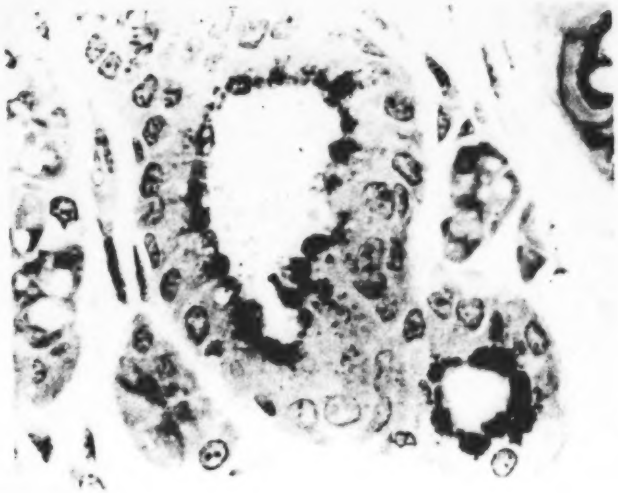

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FIG. 4. A representative tracheal section from a control rat stained with alcian blue–PAS at pH 2.5, × 384. Note the staining of the submucosal gland and its acini in this section.

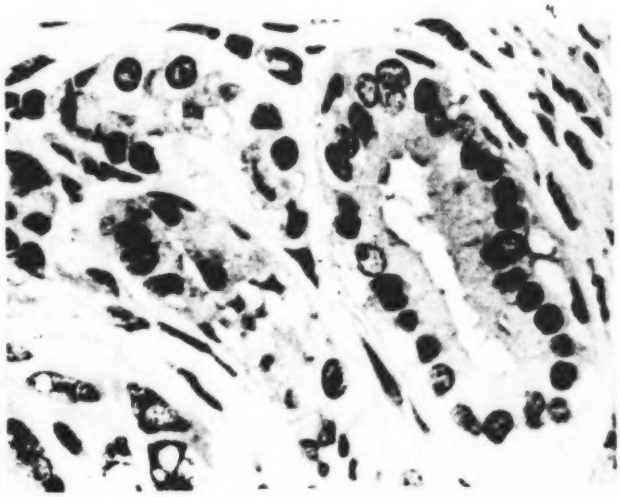

FIG. 5. A representative tracheal section from a rat exposed to 0.173 mmol/m³ of Na_2CrO_4 aerosol for 24 hr, stained with alcian blue–PAS at pH 2.5, × 384. Note the relative absence of staining within the submucosal gland and the acini.

occur frequently enough in the epithelium for any conclusions to be drawn relevant to their content of mucosubstances. The changes in the submucosal glands were striking. Stainable mucosubstances of both neutral and acidic types were almost completely absent, even though cellular necrosis was not as prevalent in these glands as it was in the surface epithelium.

We interpret the findings shown in Figs. 2–5 as follows: (i) chromate exposure (0.173 mmol/m^3) resulted in acute cell injury and cell death in the tracheal epithelium and, to a lesser extent, in the submucosal glands. There was attendant acute inflammation in the areas of necrosis, and (ii) there was a decrease in the amount of stainable mucosubstance in the tracheal epithelium and in the submucosal glands, due either to discharge of secretion granules in response to chromate exposure or to depression of mucosubstance synthesis due to acute cell injury.

DISCUSSION

Considering the nature of the assumptions inherent in the calculation of equivalent dosages of Na_2CrO_4 *in vitro* and *in vivo* by Eqs. (8) and (11), the agreement of the data of Table 2 for the two types of exposures is remarkably good. Either this agreement is fortuitous or we can conclude that contributions to the steady-state level in the tracheal fluid of a soluble substance such as Na_2CrO_4 from the aerosol cleared upward after deposition in the bronchi are negligible as compared to the quantity of aerosol directly deposited on the tracheal surface. Such a conclusion would obviously pertain only to a given aerosol particle diameter and composition. In the present case, the Na_2CrO_4 aerosol size was estimated from the nebulizer output specifications and was also measured aerodynamically with a cascade impactor. To obtain the requisite sensitivity for the latter analysis, atomic absorption spectroscopy was used to assay for chromium present upon the impactor stages. Comparison of the chromium concentration obtained by atomic absorption and the aerosol mass determined by filter sample weights showed the sodium chromate aerosol to be in the form of the decahydrate, even though the form actually weighed out into the nebulizer was the tetrahydrate.

The apparent deposition fractions as given in Table 2 are consistent with the hypothesis that the *in vivo* deposition of inhaled sodium chromate aerosol leads to the same quantitative effects as the equivalent *in vitro* exposure of tracheal explants. The numerical differences between the two fractions are probably more related to uncertainties in the calculations than real. Both values are in the range of 0.22 to 0.79% for tracheal (plus laryngeal) deposition in rats reported by Raabe *et al.* (1977) for insoluble monodisperse aerosols of 1.14- and 2.19-μm aerodynamic (resistance) diameters, respectively. Deposition was determined after a 20-min exposure so that some clearance of tracheally deposited aerosol to the laryngeal region could occur. Since the sodium chromate particles are somewhat deliquescent, their increase in aerodynamic size in the humid respiratory airways of the nose should be expected, leading to higher tracheal deposition. Raabe *et al.* (1977) reported a tracheal plus laryngeal deposition of 3.6% for 3.15-μm aerodynamic (resistance) diameters with 0.2% associated with the trachea and 3.4% associated with the larynx.

In summary, Na_2CrO_4 aerosols delivered to rats *in vivo* elicited changes in the metabolism of their tracheal explants, evaluated as rate of secretion of mucus glycoproteins in culture. The quantitative data thus obtained were compared with comparable data obtained from experiments done completely *in vitro*, wherein rat tracheal explants were incubated in tissue culture medium containing Na_2CrO_4. By the use of an appropriate equation, derived herein, we can calculate equivalent concentrations of Na_2CrO_4 aerosols *in vivo* and of Na_2CrO_4 solutions *in vitro*. In addition, qualitative morphological and

histochemical techniques were applied in both systems to demonstrate that similar pathological processes were being elicited. Importantly, the data presented in this paper, especially those of Table 2, document that, at least for one water-soluble pollutant (Na_2CrO_4), quantitative measurements of toxicity that can be performed rapidly in a relatively simple *in vitro* assay accurately mirror the actual acute toxicity of the pollutant at an equivalent dose *in vivo*. Such methods should prove extremely useful for preliminary evaluations of the toxicity of a wide variety of soluble pollutants that might be anticipated to occur in aerosols arising from the combustion of coal (Fisher *et al.*, 1977) or from a wide variety of industrial processes (Gafafer, 1964).

The absorption and excretion of zinc chromate after inhalation exposures in rats were reported since this paper was submitted (Langård *et al.*, 1978).

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